



Performance of Anaerobic Co-digestion of Pig Slurry with Pineapple (*Ananas comosus*) Bio-waste Residues

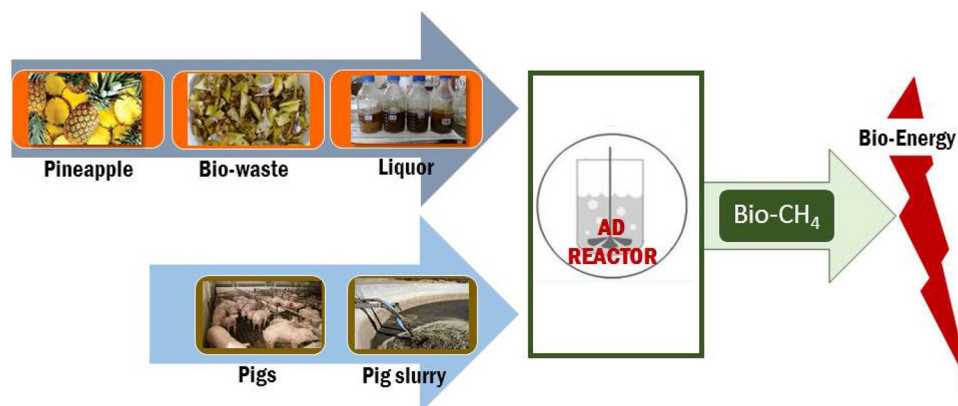
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Abstract

Agro-food industries produce large amounts of bio-waste, challenging innovative valorisation strategies in the framework of circular economy principles. Anaerobic digestion technology is an interesting route to stabilise organic matter and produce biogas as a renewable energy source. This paper aimed to study the optimal performance conditions for anaerobic co-digestion (AcoD) of pig slurry with pineapple (*Ananas comosus*) peel bio-waste. The anaerobic digestion (AD) trials were performed at lab scale, in a continuous stirred reactor, for 16 days' hydraulic retention time in mesophilic conditions (37 ± 1 °C). Three hydraulic retention time were performed, one for the reference scenario (T_0) and two for AcoD trials (T_1 , T_2). Feeding mixtures (20:80; v:v) of pineapple peel liquor and pig slurry, with an OLR of 1.46 ± 0.04 g TVS $L^{-1}_{\text{reactor}} \text{ day}^{-1}$ were used during AD/AcoD trials, presenting high values for soluble chemical oxygen demand and C/N ratio. This operational conditions highlight bioenergy recovery up to $0.58 \text{ L CH}_4 \text{ g TVS}_{\text{added}}^{-1}$, in comparison with that obtained with pig slurry substrate ($0.31 \text{ L CH}_4 \text{ g VS}_{\text{added}}^{-1}$). The AD performance showed a total volatile solids and chemical oxygen demand removal efficiency of 23% to 47% and 26% to 48%, comparing T_0 with the average of T_1 and T_2 , respectively. The digester stability, evaluated by specific energetic loading rate, was below the limit (0.4 day^{-1}) throughout the trials. Pig slurry co-digestion with pineapple peel liquor seems to be a promising approach for potential bioenergy recovery.

Graphic Abstract



Keywords Biogas · Waste management · Pineapple · Pig slurry · Circular economy

Abbreviations

AcoD Anaerobic co-digestion
 AD Anaerobic digestion

C/N Carbon/nitrogen ratio
 GPR Gas production rate
 HRT Hydraulic retention time
 IA Intermediate alkalinity
 OLR Organic loading rate
 PA Partial alkalinity

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PPL	Pineapple peel liquor
PS	Pig slurry
SCOD	Soluble chemical oxygen demand
SELR	Specific energetic loading rate
SGP	Specific gas production
SMP	Specific methane production
TA	Total alkalinity
TCOD	Total chemical oxygen demand
TKN	Total Kjeldahl nitrogen
TOC	Total organic carbon
TS	Total solids
TVS	Total volatile solids
TSVS	Total suspended volatile solids

Statement of Novelty

- Methane yield upgrade in AcoD trials, using a feedstock (80% pig slurry:20% pineapple peel liquor v:v);
- Synergetic effects between C/N ratio, SMP and AD efficiency;
- Valorisation of an agro-food bio-waste to bioenergy recovery (Bio-CH₄).

Introduction

Livestock production is an important industry sector considering economic and global food security aspects [1]; however, this growth in activity has led to enormous environmental impacts, which have started to threaten the resilience of natural ecosystems [2]. The world production of pig meat increased by 29% between 2000 and 2016, and it is expected to increase 9.5% more by 2026, to reach 127.5 million tons [3]. Livestock waste management is a major challenge due to its pollutant load [4], and swine production already generates a high volume of waste with high pollutant concentrations [5].

In addition, agro-food industries, like the food fruit processing industry, generate large amounts of bio-waste (e.g. peels, barks). Pineapple (*Ananas comosus*) is the third most produced tropical fruit, after banana and mango [6]. It belongs to the Bromeliaceae family and has a total cultivated area of 1.02 million hectares, with a global production of 24.8 million tons year⁻¹, resulting in great quantities of bio-waste (peels and rags) [7].

The most innovative environmental management strategy is the potential bioenergy recovery of this bio-waste generated in the food processing chain, as feedstocks for promoting the circular bioeconomy concept. Based on this approach, anaerobic digestion (AD) technology is an interesting route to combine these two substrates (with different

physical/chemical characteristics), to produce renewable biofuels (Bio-CH₄) and improve the stabilisation of pig slurry organic matter, respectively [8]. The biogas obtained can be used to produce electric and thermic energy, to inject into the natural gas network (when purified), or as a biofuel in transport, among other applications [9, 10].

The production of this renewable biofuel can bring many social and environmental benefits, such as a reduction of the organic matter content; reduction of greenhouse gas emissions; global warming mitigation; and a reduction of dependence on fossil fuels, among others [11]. Besides that, the solid fraction of the digestate produced during AD can be applied as a biofertiliser in agricultural practices [12].

Different researchers have studied the use of different co-substrates in the AD process in order to increase the methane content in biogas; e.g. agro-food fruit bio-waste (banana peels, citrus peels) [13, 14], biomass from energy crops (*Cynara*, elephant grass) [15, 16], and agricultural and municipal waste [17]. Since pineapple peel is rich in carbohydrates (cellulose, hemicellulose and free sugars) it is a potential co-substrate in AD [18]. In addition, those substrates increase the stability of the AD process, enhancing the nutrient balance, ameliorate microorganisms' synergistic effects; and help to mitigate greenhouse gas emissions [19].

The aim of this study was to assess the influence of pineapple peel liquor as a co-substrate to enhance AD of pig slurry from the fattening/finishing phase, in a continuous stirred reactor at mesophilic conditions (37 ± 1 °C), and a hydraulic retention time (HRT) of 16 days. For these purposes, the liquor from pineapple peel used as co-substrate was fully characterised (chemically, physically and energetically), and the Bio-CH₄ content evaluated in AD and AcoD trials.

Experimental Sections

Materials

Pig Slurry

Pig slurry (PS) was provided by a swine livestock facility located 90 km from Lisbon in the Alentejo region, Portugal, with a total area of 1050.320 m² and capacity for 900 sows, with 3924 fattening places. The production cycle consists of: gestation–maternity–lactation; post-weaning nursery; and fattening/finishing. The slurry management system of the farm includes a storage tank, solid–liquid separation of slurries, and a lagoon system. Samples were collected from the fattening slurry storage tank for utilisation in trials. The samples had remnants of grains and coarse material, so the slurries were sieved with a strainer with a mesh size of 2 mm to remove

these residues. After sieving, the remaining liquid fractions were stored at 4 °C.

Pineapple peel

Pineapple residues from 18 fruits were collected from the ‘Time Out Market Lisboa’, Portugal where ‘Compal Frutológica’ makes on the spot and serves directly to the customer 100% juices of fruit selected from the country’s best growers. The fresh residues were separated into two different fractions, pineapple crown (PC) and pineapple peel (PP), weighed and dried in an oven at 80 °C, until achieving a constant weight. Further, the PP residues were milled in a knife mill (Retsch SM 2000) with an output sieve of 6 mesh and stored in a plastic bag for future use.

Methods

Pineapple Peel Liquor

The PP residue was thermal-hydrolysed using a liquid to dried solid ratio of 10:1 for 5 min at 120 °C and 1.08 bar.

The pre-treatment was conducted in an autoclave (Uniclave 88) with capacity for 24 flasks of 1 L and a power of 6 kW. Afterwards, the pineapple peel liquor (PPL) was sieved with a strainer with a mesh size of 2 mm to extract solid residues. The volume of the liquid fraction was registered to calculate the liquor efficiency recovery reported as a percentage of fresh pineapple fruit weight. The PPL was stored at 4 °C for utilisation as co-substrate, while the solid fraction was disposed of.

Chemical and Thermal Characterisation

Chemical analysis of the PP was performed according to standard methods: ash content by TAPPI 211 om-02, and extractives content by successive extraction with dichloromethane, ethanol and water (TAPPI 204 cm-97). Klason lignin was quantified using TAPPI 222 om-02, and the acid-soluble lignin determined by absorbance at 205 nm in the hydrolysate (TAPPI UM 205 om-93). The holocellulose and its α -cellulose and hemicellulose fractions were analysed according to Rowell [20]. Mineral composition (Na, K, Mg, P, S, Fe, Cu, Zn, Mn, B) was determined using a flame atomic absorption spectrophotometer (AnalytikJena multi EA 4000), and the higher heating value (HHV) was determined based on ASTM D-2015–66.

Experimental Setup

Feeding Mixtures

During the reference scenario (T_0), the AD reactor was only fed with PS from the fattening/finishing phase, after steady state conditions had been achieved. For the co-digestion

experiments, two trials (T_1 and T_2) and a PPL to PS ratio of 20:80 were used (Table 1).

Feeding Mixture and Digestate Characterisation

The following feeding mixture parameters were characterised in accordance with the Standard Methods for the Examination of Water and Wastewater [21]: pH, electrical conductivity (EC); total solids (TS); total volatile solids (TVS); total volatile suspended solids (TVSS); total chemical oxygen demand (TCOD); soluble chemical oxygen demand (SCOD) and total Kjeldahl nitrogen (TKN). Total organic carbon (TOC) was determined by following the method described by Cuetos et al. [22], and compared with some measurements obtained using a carbon analyser (TOC, AnalytikJena Multi EA 4000), by performing combustion of the sample at 1200 °C. The C/N ratio was calculated by dividing the TOC by the TKN. Total alkalinity (TA) and partial alkalinity (PA) were determined by a titration method at pH 4.3 and at 5.75, respectively, and the intermediate alkalinity (IA) by the difference between TA and PA [21].

Lab-Scale Experiment

The anaerobic co-digestion (AcoD) experiment was performed during three trials (T_0 , T_1 and T_2) in a mesophilic temperature range (37 ± 1 °C) with an HRT of 16 days. The reactor was a CSTR (continuous stirring tank reactor) with a working volume of 4.8 L, controlled by computer software. The temperature inside the reactor was maintained by a heating system and recorded along the trials. The feeding mixture was homogenized by a mechanical stirrer (model VELP Scientifica, 50 rpm, 60 W) and added to the digester using a peristaltic pump (model Watson Marlow, 120 rpm). A flow-meter was used to control the biogas production (model MilliGascounter MGC-1 V3.0, Ritter, Germany) as illustrated in Fig. 1. Temperature, pH and EC of the inlet and outlet flows and biogas production were measured daily.

Operational Parameters

During the experiment, several parameters were determined in order to study the AD process: gas production rate (GPR);

Table 1 Experimental assay setup configuration

Experimental trials	HRT (days)	Temperature (°C)	Feeding mixture (PPL:PS v:v; %)
T_0 —reference trial	16	37 ± 1	0:100
T_1 —first co-digestion trial	16	37 ± 1	20:80
T_2 —second co-digestion trial	16	37 ± 1	20:80

organic loading rate (OLR); specific gas production (SGP) and specific methane production (SMP). Biogas quality (in terms of CH_4 and CO_2) was measured on a weekly basis, using an LMSxi Multifunction Landfill Gas Analyser. The digester was continuously monitored by on-site pH probe and by periodical analysis of TA and PA. To evaluate the reactor stability during the trials, the indicator IA/PA proposed by Astals et al. [23] and the specific energetic loading rate (SELR) [24] were determined. Regarding the reactor performance, TVS and TCOD removal efficiency were calculated at the end of each trial and correlated with the SMP values achieved.

Results and Discussion

The summative chemical and mineral composition of PP is presented in Table 2. Ash represented 4.1% of the PP, while total extractives accounted for 56.2% (mainly constituted by polar compounds attained by ethanol (38.8%) and water extractions (16.5%)), total lignin 7.1% and holocellulose (hemicelluloses + cellulose) 33.7%.

The ash content in PP is much lower than in other fruit peels such pawpaw (10.2%), banana (12.4%) or pomegranate (6.1%), but higher than in apple (1.4%) and mango (3.2%) peels [25]. Pardo et al. [26] studied different fractions from pineapple residues and determined that the ash represented 3.0% in the pulp, 7.4% in the leaf bracts, 1.5% in the shells and 1.3% in the core.

Table 2 Summative chemical composition and mineral composition of pineapple peel

Component (Mass fraction, %)		Mineral content (mg per 100 g DM)	
Ash	4.1	Na	16.4
Total extractives	56.2	K	17,043
Dichloromethane	0.9	Mg	85
Ethanol	38.8	P	153
Water	16.5	S	137
Total lignin	7.1	Fe	4.4
Klason lignin	5.6	Cu	0.8
Soluble lignin	1.5	Zn	1.6
Holocellulose	33.7	Mn	4.4
α -Cellulose	10.9	B	0.7
Hemicellulose	22.8		

DM dry matter

Regarding the total lignin content, the value attained here (7.1%) was lower than the 13.8% and 10.0% reported for leaf bracts and shell fractions, but slightly higher than the 5.8% for the core fraction [26]. The same study revealed substantially more carbohydrate content in pineapple residues, ranging from 53.0% (core) to 69.2% (shell) when compared to the value reported here (33.7%). Lukitawesa et al. [27] found 19.0% carbohydrate content in citrus peel.

PP is rich in K (17,043 mg 100 g⁻¹) and P (153 mg 100 g⁻¹). Morais et al. [28] reported 1349.5 mg per 100 g for K, and Romelle et al. [25] 6.5 mg per 100 g for Zn and 5.3 mg per 100 g for Mn.

The proximate analysis and calorific value of PP used in this experiment are shown in Table 3. The mean HHV was 17.7 MJ kg⁻¹ that is in the range of values reported for other fruit peels (16.2 to 19.6 MJ kg⁻¹) [29]. The proximate analysis of PP showed a mean of 66.0% volatile matter. Lukitawesa et al. [27] reported 23.1% for volatile solids and Carvalho et al. [30] 45–51 g/L for citrus peels (dry basis).

Feeding Mixture and Digestate Characteristics

The results obtained during the experimental trials are presented in Table 4.

Table 3 Proximate analysis and calorific value of pineapple peel

Moisture (% dry basis)	13.0
Ash (% dry basis)	4.1
Volatile matter (% dry basis)	66.0
Fixed carbon (% dry basis)	16.9
High calorific value (MJ kg ⁻¹)	17.7

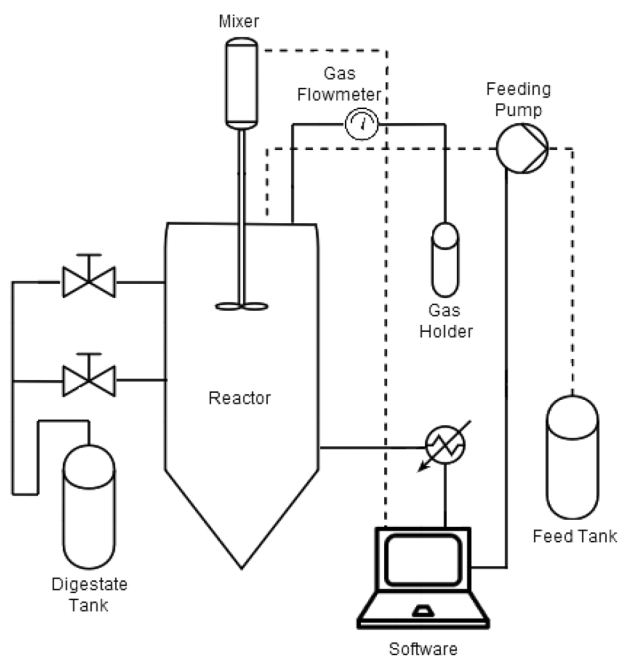


Fig. 1 Schematic diagram of biogas unit used for experimental assays

Analysing Table 4, and according to trial T₀, the PS inlet values showed an average pH of 7.6 ± 0.1 , which is in accordance with the range expected for this type of substrate [31] and referred to as ideal in the literature for AD processes [32, 33]. The slight decrease of pH average value (7.2 ± 0.2), during AcoD is probably due the presence of biodegradable sugars. The TVS/TS ratio increased, from the reference trial (T₀) to the co-digestion trials (T₁ and T₂) (64.5% to 71% and 72%) respectively. During the AcoD trials (T₁ and T₂), the feeding mixture was prepared by combining PPL and PS (20:80, v/v PPL:PS) prepared under the selected conditions as previously described. This procedure improved the availability of organic matter as shown by the SCOD/COD ratio twice that obtained during T₀ (Table 4). This aspect is very relevant as the low solubilisation degree of PS organic matter is responsible for the low methane yields generally achieved. The addition of the pineapple peel liquor (PPL) originated a decrease of the mixture nitrogen compounds, more specifically, a 44% decrease of TKN, from T₀ to T₁ and T₂. Due to the decrease of TKN present in the mixture, the C/N ratio improves by doubling the value from the initial trial (T₀) value to the third trial, T₂. The improvement of this value generates higher methane yields in the trials following T₀, which contributes to balancing the feedstock composition, allowing better performance of the AD biotechnological process [34].

Anaerobic Digestion Stability and Performance

In order to assess the process performance and stability, different AD experiments with PS as a mono-substrate and PS with addition of PPL as feedstock's for co-digestion were performed at similar OLR and hydraulic retention times (HRT). Figure 2 shows the average gas production rates

(GPR) correlated with the OLR's applied in the reference scenario (T₀) and in the co-digestion trials (T₁ and T₂).

These data allow seeking the influence of the co-substrate incorporation over the AD process and biogas production. The average daily biogas production rate during the reference trial was approximately $0.69 \pm 0.15 \text{ L L}^{-1}\text{reactor}$, in comparison with the values achieved for T₁ and T₂ ($1.15 \pm 0.03 \text{ L L}^{-1}\text{reactor}$ and $1.17 \pm 0.03 \text{ L L}^{-1}\text{reactor}$), respectively. This means an increment of 67% in the GPR from the reference trial to the first co-digestion trial, and 70% in the last trial. These results are also aligned with those reported by Duan et al. [35] for single treatment of PS, attending to the HRT of 16 days set in the study presented. The results obtained in the CoAD trials with PPL showed an improvement of gas productions rate in comparison with other studies referred in the literature [36].

The digestate pH and SMP were monitored and correlated with IA/PA indicator and SELR to evaluate the stability of AD/AcoD process (Fig. 3).

The operational parameters monitored during the trials, presented in Table 5, clearly illustrate the enhancement of bioconversion during the co-digestion process. To corroborate this statement, we can refer to the increase in GPR (around 70%) between the reference trial and the co-digestion trials.

The SGP (Table 5) in the reference trial was 0.44 L g VS^{-1} ; it was 0.80 L g VS^{-1} during the co-digestion trials (that corresponds to an increase of almost 82%). The same happened for the SMP, with an increase of almost 84% (from 0.31 ± 0.05 to $0.57 \pm 0.07 \text{ L g VS}^{-1}$).

During the reference trial, the reduction in TVS was only 23%; in the co-digestion trials it was almost 47%, which means an increase of 103%. The reduction in TCOD in the

Table 4 Feeding mixture and digestate characterisation

Characteristics	T ₀ (0:100, v/v PPL:PS)		T ₁ (20:80, v/v PPL:PS)		T ₂ (20:80, v/v PPL:PS)	
	Influent	Digestate	Influent	Digestate	Influent	Digestate
pH	7.6 ± 0.1	7.8 ± 0.1	7.2 ± 0.2	7.8 ± 0.04	7.1 ± 0.1	7.9 ± 0.03
EC (mS cm ⁻¹)	15.1 ± 6.6	17.9 ± 2.1	18.1 ± 0.4	22.9 ± 1.2	18.1 ± 0.3	21.2 ± 0.4
TS (g L ⁻¹)	37.2 ± 0.03	30.3	32.6 ± 0.02	22.2	31.8 ± 0.01	21.1
TVS (g L ⁻¹)	24.0 ± 0.02	18.4	23.2 ± 0.02	12.3	22.9 ± 0.01	12.1
TVS/TS (%)	64.5	60.7	71.2	55.4	72.0	57.3
TVSS (g L ⁻¹)	–	8.12	–	8.81	–	8.52
TCOD (g L ⁻¹)	38.4	28.3	47.8	25.0	46.1	24.2
SCOD (g L ⁻¹)	10.3	7.0	24.9	6.7	24.0	5.8
SCOD/TCOD (%)	26.8	–	52.1	–	52.1	–
TOC (g L ⁻¹)	13.9	–	13.5	–	13.4	–
TKN (g L ⁻¹)	2.6	–	1.5	–	1.4	–
C/N	5	–	9	–	10	–
OLR (g TVS L ⁻¹ d ⁻¹)	1.50 ± 0.02	–	1.45 ± 0.02	–	1.44 ± 0.01	–

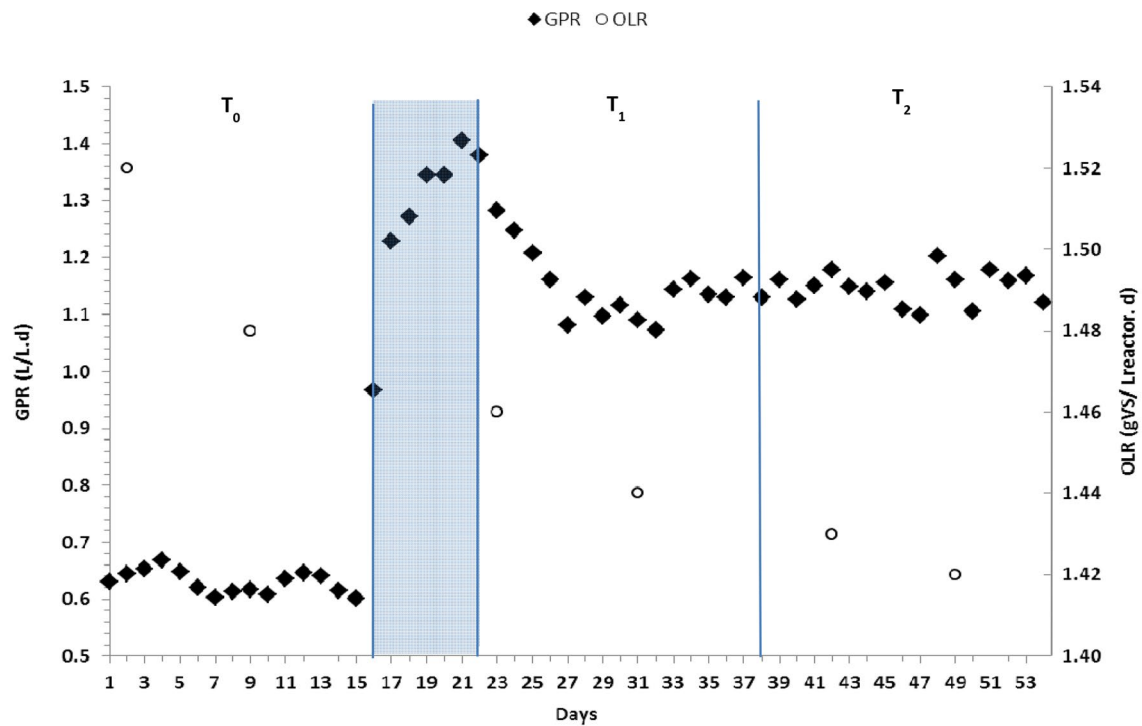


Fig. 2 Variation of the gas production rate and the organic loading rate in the reference scenario (T0) and in the co-digestion trials (T1 and T2)

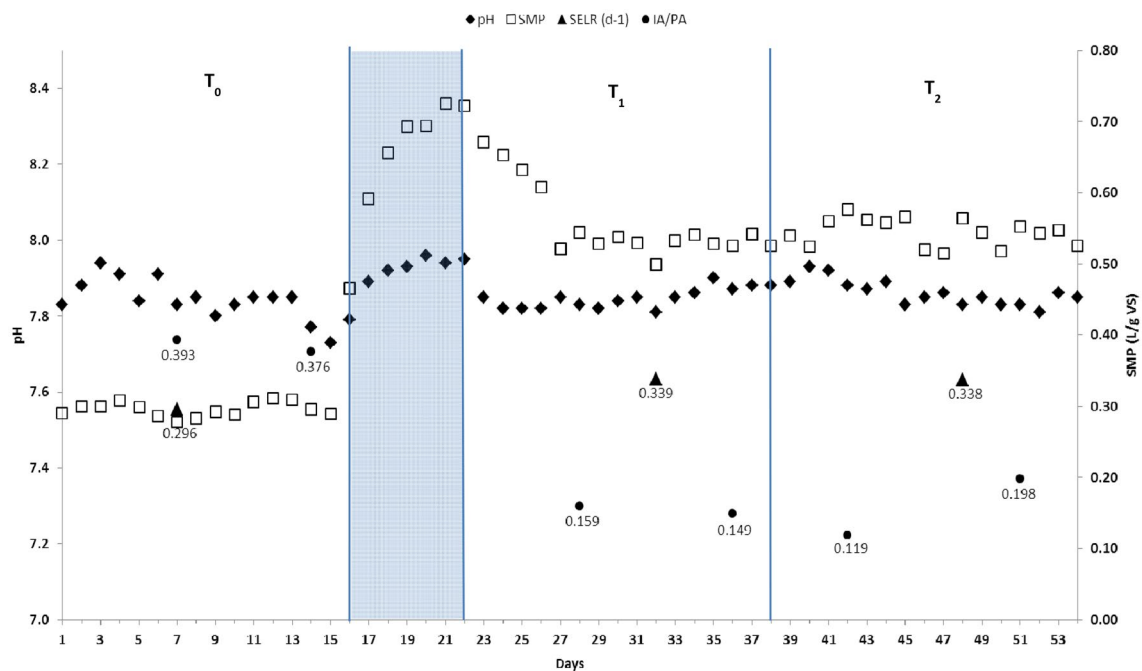


Fig. 3 Influence of the different feedstock composition over the digester control parameters: pH, SMP, IA/PA ratio and SELR

reference trial was 26%; in the following trials it was 48%, representing an increase of 82% (Table 5).

The stability of an AD process, in terms of VFA accumulation and alkalinity, depends mainly on the type of substrate used and relative percentages of co-substrate. The alkalinity

Table 5 Operational parameters during the AcoD trials

Operational parameters	T ₀ (0:100, v/v PPL:PS)	T ₁ (20:80, v/v PPL:PS)	T ₂ (20:80, v/v PPL:PS)
GPR (L L ⁻¹ day ⁻¹)	0.69 ± 0.09	1.15 ± 0.08	1.17 ± 0.03
Biogas quality (% CH ₄)	72	69	68
SGP (L _{Biogas} g VS ⁻¹)	0.44 ± 0.06	0.80 ± 0.06	0.80 ± 0.02
SMP (L _{CH₄} g VS ⁻¹)	0.31 ± 0.05	0.57 ± 0.07	0.54 ± 0.02
TVS reduction (%)	23.3	47.0	47.2
TCOD reduction (%)	26.3	47.8	47.5
TA (mg CaCO ₃ L ⁻¹)	7886	12,080	10,786
PA	5730	10,468	9306
IA	2156	1612	1480
IA/PA ratio	0.376 ± 0.012	0.154 ± 0.007	0.159 ± 0.056
SELR (day ⁻¹)	0.296	0.339	0.338

ratio contributes for the buffer capacity of the bioreactor content [37]. A decrease in the buffering capacity caused by the accumulation of VFAs comes earlier than the pH decrease. Therefore, the IA/PA ratio is a reliable parameter to monitoring AD process imbalance. The recommended IA/PA ratio for a stable process is below 0.4 [38].

The parameters analysed to evaluate the AD reactor stability, during the experimental trials were IA/PA ratio and SELR. The IA/PA values observed during the AD/AcoD trials (0.376 ± 0.012 ; 0.154 ± 0.007 and 0.159 ± 0.056) were always below than the lower limit advisable for assuring stable process conditions [38]. According to Evans et al. [24], the limit value for SELR is 0.4 day^{-1} . A SELR value higher than 0.4 day^{-1} indicates instability among the microbial consortia biomass and feeding mixture loading. In this experiment, the reference trial SELR value was 0.296 day^{-1} , and during the co-digestion trials it was 0.339 day^{-1} , indicating the stability of the AD process and the possibility of the OLR increment.

Conclusions

The results of this study highlight the potential use of PP bio-waste as a biomass source for bioenergy recovery. A stable process operation was observed at an OLR of up to $1.50 \pm 0.02 \text{ g VS L}^{-1} \text{ day}^{-1}$, with a highest biogas rate production achieved of $1.17 \pm 0.03 \text{ L L}^{-1} \text{ day}^{-1}$ and an HRT of 16 days. The highest efficiency rate regarding the specific methane yields was $0.57 \pm 0.07 \text{ L CH}_4 \text{ g VS}^{-1}$, at an OLR of $1.45 \pm 0.02 \text{ g VS L}^{-1}$, due to PPL addition in AcoD trials. Biogas production from PS (80%) with PPL (20%) in the CSTR pilot scale AD system and an HRT of 16 days revealed high efficiency: biogas yield of $0.80 \text{ L g TVS}_{\text{added}}^{-1}$, TVS and COD removal of 47% and 48%, respectively, were achieved in the AcoD trials (T₁ and T₂). Co-digestion of PS and pineapple waste has a synergistic effect, which improves

the biodegradation of feedstock. This effect resulted in a higher methane yield than input of PS alone to the digester. In particular, regarding Portuguese pig livestock units that face a high demand for the waste management of manure, these results can contribute to an increase in the sector's sustainability. Through the adoption of slurry segregation, using a slurry storage tank from the fattening/finishing phase without solid/liquid separation, co-digestion with PPL could be a very effective method to upgrade the performance of AD process technology.

PP bio-waste residue is a promising AcoD substrate that contributes to the valorisation of agro-food bio-waste for bioenergy recovery (Bio-CH₄) in the framework of circular economy principles.

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